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The Histology of the Eye of the Cave Characin, *Anoptichthys.*

E. B. GRESSER & C. M. BREDER, JR.

Department of Ophthalmology, New York University College of Medicine, New York Aquarium.

(Plates I-III).

INTRODUCTION.

The blind cave characin, *Anoptichthys jordani* Hubbs & Innes, described in 1936, represents the first of the order Heterognathi to show loss of ocular structure. The Eventognathi and Nematognathi both long have been known to have cave representatives with defective or absent vision. Since cave fishes are not exactly numerous nor spread through many of the orders of fishes, it is interesting to find species in each of the major divisions of the Ostariophysi. In a list presented by Hubbs (1938) in which he names all blind fishes and cave fishes, whether blind or not, exclusive of deep sea forms, 20 belong to the Ostariophysi, while all the rest contribute only 27. Of the former, 19 are derived from barbeled forms, and, as Hubbs points out, this may well have to do with their relatively numerous presence in caves.

EYE STRUCTURE.

The eyes of *Anoptichthys jordani* are somewhat translucent and depressed below the surface of the ectoderm. A pigment layer is apparent by direct examination, this layer forming, as far as is evident by the eye, a cyst wall without an anterior opening.[1] As will be shown later, this is evidenced microscopically to be an intact cyst without a pupillary space.

In a fish 56 mm. long, the gross measurements of the optic organ are 1.5 mm. vertically, 1.5 mm. horizontally, and 1.2 mm. antero-posteriorly. The eye is definitely below the level of the skin and is over-lapped by folds of epidermis which in some specimens continue inwardly to form a solid stalk of epithelial tissue and in others an invagination of epithelium forming a blind sac. In both formations the epithelial column is directly in contact with a solid mass of condensed mesodermal tissue corresponding to the cornea. The attachment of this epithelial column to the corneal layers is within the corneal surface area and not beyond. Intervening from all sides around the stalk is a loose mesynchymal structure.

In general the eye has recognizable definitive layers, although of poor development. The external musculature is present and an optic nerve has an outline. The primitive optic vesicle without question has undergone its first invagination and at this level apparently ceased to differentiate beyond a pigment layer and a retinal layer. At this point it may be well to acknowledge that evidence set forth below shows the primary vesicle after its invagination to be definite in charactèr by the presence of the pigment

[1] The distal portion of the eye is here referred to as anterior.

epithelium and several layers of retinal elements, although there has taken place a degenerative change in which the edges of the vesicle have fused, with poor attempts at the formation of a rod and cone layer and absence of an outer nuclear layer. It is conjectural at this stage of development whether the force—genetic, hormonal or intracellular metabolic control—has failed to stimulate the overlying epithelial cells to the formation of lenticular tissue, or perhaps the epidermal column does represent this attempt and is shown by its failure to progress beyond the stage of forming a secondary vesicle. Whatever the answer may be, whether the eye stops in development because no lens develops and produces in turn the fusion of the poorly differentiated primary vesicle into a cyst, or vice versa, the eye has not progressed to any stage wherein vision can be served.

The cartilaginous-walled orbital cavity contains six extraocular muscles that probably are functionally useless, judging from the weak striations in the fibers.

The outer mesodermal layers are the best developed, a well marked cartilaginous sclera, cup-shaped as is characteristic of piscine eyes, with a posterior aperture for the entrance of an optic nerve and anteriorly replaced by a laminated translucent fibrous tunic representing the interstitial tissue of the cornea. It is apparent from what has been said above that the primary vesicle, although differentiated and invaginated, still forms a complete cyst, thereby precluding the presence of a pupillary opening or permitting the development of iris tissue as such. Some basis, however, for the possible formation of iris tissue is present, as is evident by the pigment epithelium and in addition the extension of a layer of small blood vessels derived from the choroidal network. Between this layer and the interstitial mesoderm of the corneal analogue there is a solid mass of large, light-staining cells with a faint amount of chromatin material, mesenchymal in appearance, that is present in all the eyes examined. It is probable that this is a primitive tissue of supporting nature that would have disappeared if the eye had progressed to maturity. In an occasional eye, not in every one examined, there has been seen a well-defined cleft, unfilled with cells, that stimulates the imagination to conceive it as a possible anterior chamber, inasmuch as it possesses a definite single layer of flat lining cells.

The choroidal layer is as well defined as the sclera. A definite choroidal gland is present, with its system of blood vessels, and warrants no further description.

The retinal pigment epithelium is the best-defined of the retinal layers and throughout is a layer of irregular hexagonal cells within which are massed brown melanin pigment granules. This layer, the outer part of the optic vesicle and without question continuous with the layer of nerve elements on its inner surface, does not contain a space between the borders of invagination but is apposed to its opposite side, forming a complete cyst. In several of the eyes examined there has been further growth of the wall of the optic vesicle with the formation of subsidiary cysts within the main body, each lined with an aborted type of retina. Of the usual stratification of the retina that is discernible, the inner nerve fiber layer is weak and thin throughout, even at the entrance of the optic nerve where it might be expected to have a thickness of fibers. The ganglion cell layer is in the main a single layer of cells which is neither continuous throughout nor well developed. The inner plexiform layer is found without obvious defects in its architecture. The inner nuclear layer is definite but lacks structural delineation in its finer distribution and in the quality of its clear arrangements. The outer plexiform layer or network is weak and thin. Both the outer nuclear layer and the layer of rods and cones show the poorest attempts at development. Where the one fraction of a tissue such as the rods or cones is not to be found, it is evident that their nuclear parts would be absent. It is not possible either from our material or present knowledge to state upon which deficiency is predicated the non-development of the elements. In several areas, islands of rods with small nuclei are found, but in which no sign of a limiting membrane between the two parts is seen. Another frequency are areas or islands of embryonal retinal cells that have a pigment mantle but cannot be differentiated as to type. The abortive attempts to form rods or cones are usually found in the more peripheral parts of the cyst wall.

Only at the center of the vesicle is there any evidence of a vitreous body. The retinal tissue cyst walls in most areas are apposed and only at the mid-part are slightly separated by a faint, loose syncitium of cells representing a vitreous mass. This tissue, in turn, has nuclear elements in a faintly granular cytoplasmic matrix that stains faintly with hemotoxylin, a primitive type of vitreous.

The optic nerve has not been separated into fascicles and no intraneural bundle supporting framework has been noted. Scattered throughout the nerve stalk are large, faintly basophilic nuclei containing fine, granular material. They are mainly round, evidently nuclei of nerve cells. Their appearance and their faint cytoplasmic prolongations, which are quickly lost, suggest a nerve cell of embryonic character. Nerve fibers are few in the stalk and poorly developed.

Discussion.

A prime step in any attempt to understand the nature of these peculiarities would seem to be to determine, if possible, whether these changes are degenerative in each fish or whether they are in the nature of an arrested development. The foregoing descriptive matter would seem to indicate clearly that this species lacks vision because of a stoppage in development of the eye structures at an early stage rather than a cellular inactivity at a more advanced state.

Although we have not had very young specimens available, reports that they show more evident eye structure suggest, in the light of the foregoing, that the gross appearance of the young may be accounted for by the eye capsule being more visible from the exterior because of the thinner nature of the overlying tissue.

The more interesting question of whether the fish lost its visual equipment after entering cave waters or whether a mutation with defective vision simply found it possible to survive under such conditions, even today is a matter of speculation. An experimental approach would undoubtedly be of great value in this connection.

There is evident in the literature of blind vertebrates a marked tendency toward speculation, as is usual in cases where the evidence does not point very conclusively in any given direction. This centers about the significance of phylogenetic or ontogenetic factors, the entire subject of genetics in relation to mutations and about which came first—the cave environment or the blindness. Franz (1934) sums up the school of thought in which conditioning by darkness allegedly results in blindness by the acquired increments of steadily degenerating eyes both physically and physiologically, each generation adding its own amount of increment to the transmitted factor (or loss of factor).

Eigenmann (1900) in discussing the eye of *Amblyopsis*, also attributes the lack of ocular development to a genetic cause, based on comparison with *Forbesella* (*Chologaster*) *agassizii*, through the transmissibility of accumulated degenerative changes, stressing that the phylogenetic tendency is evident in the structural formation, although an irregular individual abiotrophy ensues almost at the start.

Since fishes living in regions of low light intensity are frequently provided with large eyes, and are found in association with a lesser number with reduced eyes, as in deep sea fishes, it should follow that at least some large-eyed forms should be found in cave environments as representing a stage in the gradual accommodation to life without light at all. Actually no such condition has been found in those forms that are true cave dwellers. Hubbs (1938) describes a new subspecies of *Cichlasoma urophthalmus, C. u. ericymba,* as having a very slightly larger eye than the other subspecies of this form. These fish are, moreover, apparently not true cave fishes but occupy the more or less open cenotes of Yucatan and the difference in eye size noted by him may have nothing to do with their habitat, especially since the variation is not nearly as great as that frequently found among closely related fishes living in open waters, and also as the Cichlidae have not produced a single blind cave form. The pigment of this fish is only slightly reduced, but more striking is the unusual development of the sensory pores about the head.

The view, on the other hand, that posits a chance mutant form with an accidental association with a cave, as is discussed by Hubbs, would seem more reasonable in the light of modern evolutionary thought. Genetic experiments crossing *Anoptichthys* with *Astyanax,* rearing several generations of *Anoptichthys* in light, an examination of the food intake of wild fish from the caves, and an assay of their vitamin A content, should go far to elucidate the concepts involved. Until at least some of these are undertaken, it would seem to be pointless to attempt further speculation.

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EXPLANATION OF THE PLATES.

(Photomicrographs by Dr. R. F. Nigrelli)

Plate I.

Fig. 1. *Anoptichthys jordani.* Mid-vertical section; ocular layers differentiated but underdeveloped, except sclera and choroid. Note absence of iris, pupil, anterior chamber and crystalline lens.

Plate II.

Fig. 2. *Anoptichthys jordani.* Mid-horizontal section. Retina lacks normal architectonics; pigment epithelium fused, forming vesicle.

Plate III.

Fig. 3. *Anoptichthys jordani.* Horizontal section at periphery. Retinal vesicle subdivided into two cystic spaces. An epithelial stalk connects the subsurface eye with epithelial surface.

Fig. 4. *Anoptichthys jordani.* Retinal pigment epithelium of normal appearance; retina lacks rods and cones, and lamination of plexiform and ganglion cell layer.